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Signed Andrew Gray

Dated 3 August 1999

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GB9815163.2

By virtue of a direction given under Section 30 of the Patents Act 1977, the application is proceeding in the name of

BRAX GROUP LIMITED
Incorporated in the United Kingdom
13 Station Road
CAMBRIDGE
CB1 2JB
United Kingdom

[ADP No. 07667546001]

Patents Form 1/77

Patents Act 1977

The Patent Office

14JUL98 E375407-2 D00068
P01/7700 25.00 - 9815163.2

13 JUL 1998

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Cardiff Road
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Gwent NP9 1RH

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

1. Your reference — 87900/JND/CH/kl

2. Patent application number *(The Patent Office will fill in this part)* — 13 JUL 1998 — **9815163.2**

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

BRAX GENOMICS LTD
13 STATION ROAD
CAMBRIDGE CB1 2JB
UNITED KINGDOM

Patents ADP number *(if you know it)*

If the applicant is a corporate body, give the country/state of its incorporation — UNITED KINGDOM

4. Title of the invention

COMPOUNDS FOR MASS SPECTROMETRY

5. Name of your agent *(if you have one)* — PAGE WHITE & FARRER

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

54 DOUGHTY STREET
LONDON WC1N 2LS

Patents ADP number *(if you know it)* — 1255003

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body
See note (d))

YES

SECTION 15(4) APPLICATION ALLOWED 16.4.99

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Description	11
Claim(s)	4
Abstract	1
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Request for preliminary examination and search (Patents Form 9/77)	-
Request for substantive examination (Patents Form 10/77)	-
Any other documents *(please specify)*	-

11. I/We request the grant of a patent on the basis of this application.

Signature: Page White & Farrer
PAGE WHITE & FARRER

Date: 13-JULY-1998

12. Name and daytime telephone number of person to contact in the United Kingdom: CHRISTOPHER HILL - 0171 831 7929

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COMPOUNDS FOR MASS SPECTROMETRY

This invention relates to methods of labelling nucleic acids and other molecules with markers that are cleavably detachable from their associated nucleic acid and that are detectable by mass spectrometry. Specifically this invention refers to improved methods of detaching mass labels from their associated nucleic acids or other molecules of interest.

PCT/GB98/00127 describes arrays of cleavable labels that are detectable by mass spectrometry which identify the sequence of a covalently linked nucleic acid probe. These mass labels have a number of advantages over other methods of analysing nucleic acids. At present commercially favoured systems are based on fluorescent labelling of DNA. Fluorescent labelling schemes permit the labelling of a relatively small number of molecules simultaneously, typically 4 labels can be used simultaneously and possibly up to eight. However the costs of the detection apparatus and the difficulties of analysing the resultant signals limit the number of labels that can be used simultaneously in a fluorescence detection scheme. An advantage of using mass labels is the possibility of generating large numbers of labels which have discrete peaks in a mass spectrum allowing similar numbers of distinct molecular species to be labelled simultaneously. Fluorescent dyes are expensive to synthesise whereas mass labels can comprise relatively simple polymers permitting combinatorial synthesis of large numbers of labels at low cost.

A feature of the mass labels disclosed in PCT/GB98/00127 is the need for linker groups that covalently link a mass marker to its corresponding nucleic acid. Preferred linker groups have a number of properties:

A good linker must permit the mass marker to be separated from its nucleic acid with high efficiency prior to detection within a mass spectrometer.

Cleavage of the label from its nucleic acid should preferably be performed in-line with a mass spectrometer, possibly after some in-line pre-fractionation step such as capillary electrophoresis. This in-line cleavage step preferably does not require a complex interface with the mass spectrometer to enable this step to occur. Ideally linkers should cleave at some

predetermined point within existing instruments without any modification to the instrument beyond changes of normal operating parameters.

Linkers should cleave under mild conditions without causing cleavage or other reactions in the associated nucleic acids hence reducing noise in the mass spectrum from nucleic acid fragmentation and ionisation.

Linkers should all cleave under the same conditions to ensure all labels can be analysed simultaneously and quantitatively.

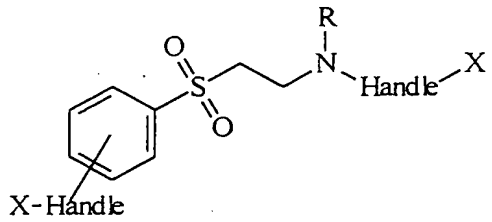
Linkers should be compatible with a wide variety of mass labels.

Linkers should preferably be attachable to a nucleic acid in multiple positions.

Linkers should be stable under conditions within conventional automated oligonucleotide synthesisers.

It is an object of this invention to provide linkers that have the desired features disclosed above which are compatible with existing mass spectrometers particularly electrospray ionisation mass spectrometry.

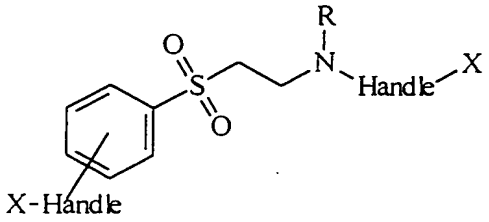
Accordingly, the present invention provides a compound having the following formula:



wherein R is an electron withdrawing substituent, one X comprises a mass marker and the other X comprises one or more nucleic acid bases, and each Handle is the same or different, being either a single bond directly attaching the X groups to the phenyl ring and the amine functionality respectively, or a divalent group capable of attaching the X groups to the phenyl ring and the amine functionality respectively.

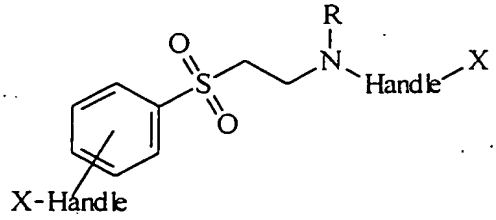
The present invention also provides a method for characterising a nucleic acid or other molecule, which method comprises identifying a mass marker by mass spectrometry, which mass marker is

relatable to a specific nucleic acid base or base sequence, or a specific atom or group in a molecule, to identify the mass marker and thereby identify the base or base sequence, or the specific atom or group, wherein the mass marker is attached to the nucleic acid or other molecule by a linker group having the following formula:



wherein R, each X and each Handle are as defined above.

The invention also provides use of a linker group having the following formula:



wherein R, each X and each Handle are as defined in any of claims 1-6, in the characterisation of a nucleic acid or other molecule, by identifying a mass marker by mass spectrometry.

The invention will now be described in further detail by way of example only, with reference to the accompanying drawings, in which:

Figure 1 shows a generic linker for use in the present invention;
Figure 2 shows a model linker used in the present invention, FT 27;
Figure 3 shows a model linker used in the present invention, FT 28;

Figure 4 depicts a schematic of the synthesis of FT27 and FT 28;

Figure 5 depicts a schematic of the synthesis of CMM267B, a linker for attachment to the 5' hydroxyl group of a nucleotide or oligonucleotide;

Figure 6 depicts a schematic for the synthesis of FT48 and FT49;

Figure 7 depicts a schematic for attachment of a linker (FT49) to a nucleobase to form FT59;

Figure 8 shows the negative ion mass spectrum of FT28; and

Figure 9 shows the probable mechanism by which F28 cleaves.

In the present invention, the nucleic acid or other molecule is preferably attached to the linker through the phenyl group and the mass marker is preferably attached to the linker through the amine functionality, but the nucleic acid or other molecule may also be attached to the linker through the amine functionality and the nucleic acid or other molecule may also be attached to the linker through the phenyl group.

The linkers used in the present invention are advantageous in that they are thermally cleavable under mild conditions. Accordingly the mass markers can be cleaved from a molecule of interest within the mass spectrometer itself.

In a first aspect, this invention provides linkers that:

- Cleave thermally under the conditions in an electrospray or thermospray ion source with a low cone voltage in an electrospray ion source.
- Are compatible with conventional oligonucleotide synthesis.

Molecules with the formula shown above meet the specification disclosed above. The linker comprises a vinylphenylsulphone group with a group substituted into the phenyl ring to permit attachment to other molecules such as nucleic acids or reporter groups for detection by mass spectrometry. The sulphone group is attached to a tertiary amine group which in turn is linked to a group to permit attachment to molecules of interest or reporter groups detectable by mass spectrometry. These linkers are stable during oligonucleotide synthesis and can be cleaved under mild conditions in an electrospray ion source or in a thermospray ion source.

Appropriate substituents that may be introduced at the R position include hydrogen, fluorine, chlorine and other halogens. Any group that is stable and which has an electron withdrawing effect is appropriate for substitution at R. A model compound substituted with a trifluoroacetate group is discussed below. Preferably substituents at R should be stable during synthesis of the marker, during incorporation of the mass label into an oligonucleotide in an automated synthesiser and under mass spectrometry.

It should be apparent to one of ordinary skill in the art that a wide variety of groups will have these properties and might be incorporated into the linker at these positions. It may also be desirable to choose substituents which change the solubility of the linker.

The linkers of this invention may have a variety of functionalities introduced at the 'handle' positions shown in the above structure.

Table 1 below shows a variety of reactive functionalities that may be reacted together to generate a covalent linkage between two entities. Any of the functionalities listed below could be introduced at the handle positions of the linker to permit the linker to be readily attached to a nucleic acid or other molecule of interest and to an appropriate reporter group for detection by mass spectrometry. It should be obvious to one of ordinary skill in the art that a reactive functionality could be used to introduce a further linking group with a further reactive functionality if that is desired.

Functionality 1	**Functionality 2**	**Resultant Linking Group**
$-NH_2$	-COOH	-CO-NH-
$-NH_2$	-NCO	-NH-CO-NH-
$-NH_2$	-NCS	-NH-CS-NH-
$-NH_2$	-CHO	$-CH_2$-NH-

$-NH_2$	$-SO_2Cl$	$-SO_2-NH-$
$-NH_2$	-CH=CH-	$-NH-CH_2-CH_2-$
-OH	$-OP(NCH(CH_3)_2)_2$	-OP(=O)(O)O-

Table 1

It should be noted that some of the reactive functionalities above or their resultant linking groups might have to be protected prior to introduction into an oligonucleotide synthesiser. Preferably unprotected ether, ester, thioether and thioesters, amine and amide bonds are to be avoided as these are not stable in an oligonucleotide synthesiser. A wide variety of protective groups are known in the art to protect linkages from unwanted side reactions.

A short alkyl linkage would be appropriate to link the mass marker to the linker although it should be apparent to one of ordinary skill in the art that a wide variety of linkers are available which can be used to link a mass marker to the tertiary amine group of the linker.

Mass markers appropriate for use with this invention are disclosed in PCT/GB98/00127 and in the co-pending applications of Page White and Farrer file numbers 87820 and 87821. PCT/GB98/00127 discloses the use of substituted poly-ethers. These are highly favoured for use as mass markers with this invention. Co-pending application of Page White and Farrer file number 87820 discloses mass labels which chelate metals ions and are thus pre-ionised before mass spectrometry. These labels have a higher sensitivity and signal to noise ratio than other mass markers. These are also highly favoured mass markers for use with this invention.

It should be noted that this invention is not limited to the mass markers disclosed in the above applications. A number of features are desirable in a molecule that is to be a good mass marker.

A marker should:

Be easily detachable from DNA.

Not interfere with enzymatic processes involved in molecular biology protocols.

Be fragmentation resistant in mass spectrometer.

Form a single ion peak in the mass spectrum.

Permit very sensitive detection.

Be easily distinguishable from background contamination, such as DNA. It should be possible to determine that a mass peak is from a mass label and not from contaminants.

Be compatible with conventional automated oligonucleotide synthesizers.

Be easy to synthesize in a combinatorial manner to minimise number of chemical steps and the number of reagents necessary to generate large number of labels.

Be compatible with existing mass spectrometry instrumentation without requiring physical modification of the instruments.

A short alkyl linkage would be appropriate to link a nucleic acid to the linker although it should be apparent to one of ordinary skill in the art that a wide variety of linkers are available which can be used to link a nucleic acid to either handle of the linkers of this invention.

Mass labels and their linkers can be attached to a nucleic acid molecule at a number of locations in the nucleic acid. For conventional solid phase synthesisers the 5' hydroxyl of the sugar is the most readily accessible. Other favoured positions for modifications are on the base at the 5' position in the pyrimidines and the 7' and 8' positions in the purines. These would all be appropriate positions to attach a cleavable mass with the linker of this invention.

The 2' position on the sugar is accessible for attachment of a linker group as is the phosphate linkage. This group can be modified to a considerable degree, including derivitisation with linkers to mass labels.

The cleavable linker used in this invention may be cleaved in the ion source of a mass spectrometer by loss of a proton followed by intra molecular rearrangements resulting in the breaking of a covalent bond in the linker. This is essentially a thermal process but can be promoted by increasing the cone voltage within the ion source of an electrospray mass spectrometer.

Examples

Synthesis of a model linker compound (FT28) that cleaves with high efficiency in ESI sources

A model linker, shown in figure (2) was synthesised (see figure (4)). This compound is identified as FT27 hereafter.

Synthesis of FT27

A solution of phenyl vinyl sulphone (1.68 g, 10 mmol) and 6-aminohexanol (1.17 g, 10 mmol) in dry methanol (20 mL) was treated with triethyl amine (0.1 mL), refluxed for two hours, and stirred at room temperature overnight. The solvent was removed under reduced pressure. The oily residue was dissolved in ethyl acetate and the solvent was evaporated to give a crystalline residue. Recrystallisation from ethyl acetate/*n*-hexane yielded 2.67 g (94 %) FT 27.

Confirmation of Identity of FT27 Product

M.p. 60–61 °C;

Calculated Atomic Composition: C 58.92, H 8.12, N 4.91; Measured Composition: C 58.91, H 8.14, N 4.89.

^{1}H NMR ($CDCl_3$) 1.30–1.55 (m, 10 H), 2.57 (t, J = 7 Hz, 2 H), 3.02 (t, J = 7 Hz, 2 H), 3.29 (t, J = 7 Hz, 2 H), 3.63 (t, J = 7 Hz, 2 H), 7.55–7.71 (m, 3 H), 7.90–7.96 (m, 2 H);

^{13}C NMR ($CDCl_3$) 25.44, 26.80, 29.69, 32,53, 43.03, 49.33, 56.02, 62.71, 128.01, 129.40, 133.84, 139.58.

Synthesis of FT28

A derivative of FT27 was synthesised (see figure (4)), identified as FT28 and shown in figure (3), in which the amine group of FT27 was protected with a trifluoroacetate group.

A solution of FT27 (855 mg, 3 mmol) in dry dichloromethane (15 mL) and triethylamine (2.5 mL, 18 mmol) was treated at 0°C with trifluoroacetic anhydride (0.85 mL, 6 mmol) and stirred for 10 min at this temperature and then for 40 min at room temperature. The reaction mixture was poured into a mixture of phosphate buffer pH 7.0 (40 mL) and methanol (80 mL) and stirred for 15 min. The organic solvents were removed under reduced pressure and the remaining aqueous residue was treated with ethyl acetate. The organic phase was separated and washed with aqueous solutions of 5 % sodium bicarbonate, 10 % citric acid and water. The organic phase

was dried with sodium sulphate and the solvents were removed under reduced pressure. The residue was purified by flash chromatography on silica gel with ethyl acetate/*n*-hexane (3:2) as eluent to afford 1.07 g (94 %) of FT28 as a waxy solid.

Confirmation of Identity of FT28 Product

^{1}H NMR ($CDCl_3$) 1.30–1.48 (m, 4 H), 1.50–1.68 (m, 4 H), 3.30–3.48 (m, 4 H), 3.65 (m, 2 H), 3.75 (t, J = 7 Hz, 2 H), 7.56–7.73 (m, 3H), 7.90–7.94 (m, 2H).

Synthesis of a linker for attachment to the 5'hydroxyl of a nucleotide or oligonucleotide

FT28 (0.306g, 0.80mmol) was co-evaporated three times with freshly distilled THF and then dissolved in THF (1.5ml). The solution was stirred under argon and diisopropylethylamine (0.45g, 0.6ml, 3.5mmol) was added. This was followed by the dropwise addition of 2-cyanoethyl-N,N-diisopropylchlorophosphoramidite (0.2g, 0.2ml, 0.85mmol) and the reaction was stirred for one hour after which time Thin Layer Chromatography showed the reaction to be complete. The mixture was diluted (DCM), washed ($KCl_{(aq)}$), dried (Na_2SO_4) and then evaporated to dryness. The residue was dissolved in DCM (2ml) then purified by precipitation into dry ice/acetone cooled hexane. The residue was dissolved in acetonitrile (1ml) and the solution was passed through a Gelman aerodisc (0.45 μm) then evaporated to dryness. The residual foam was co-evaporated several times with DCM before being dried in a vacuum desiccator over P_2O_5. This gave CMM267B (0.35g, 75%) as a white foam. $Rf_{CMM267B}$ = 0.79. Rf_{FT28} = 0.43, DCM/EtOAc 1:1, U.V.

Synthesis of a linker for attachment to a nucleobase (FT49)

A model linker, shown in figure (6) was synthesised via an intermediate identified as FT48. The model linker compound is identified as FT49 hereafter.

Synthesis of FT48

A solution of phenyl vinyl sulphone (1.68 g, 10 mmol), propargylamine (605 mg, 11 mmol) and triethylamine (0.1 mL) in methanol (20 mL) was refluxed for 2.5 hours. The solvent was removed under reduced pressure to afford 2.23 g (100 %) FT48 which was used without further purification.

Confirmation of Identity of FT48

^{1}H NMR ($CDCl_3$) 1.74 (br s, 1 H), 2.20 (t, J = 3 Hz, 1 H), 3.09 (t, J = 7 Hz, 2 H), 3.32 (t, J = 7 Hz, 2 H), 3.40 (d, J = 3 Hz, 2 H), 7.55–7.71 (m, 3 H), 7.92–7.96 (m, 2 H).

Synthesis of FT49

A solution of FT48 (2.0 g, 9 mmol) in dichloromethane (35 mL) was treated with triethylamine (2.5 mL), cooled to 0°C and subsequently treated with trifluoroacetic anhydride (2.3 g, 11 mmol). After stirring for 1 hour 40 min at this temperature the reaction was quenched with a saturated aqueous solution of ammonium chloride. The organic pase was separated, washed twice with water, dried with sodium sulphate, and evaporated to dryness under reduced pressure. The residue was purified by flash chromatoghraphy on silica gel with *n*-hexane/ethyl acetate (3:1) to furnish 2.77 g (96 %) FT49 as a colourless oil.

Confirmation of Identity of FT48

^{1}H NMR ($CDCl_3$) 2.30–2.40 (m, 1 H), 3.50 (m, 2 H), 3.93 (m, 2 H), 4.28 (m, 2 H), 7.54–7.76 (m, 3 H), 7.85–7.96 (m, 2 H).

Attachment of a linker to a nucleobase (FT59)

A solution of 5'-(4,4'-dimethoxytrityl)-5-iodouridine (656 mg, 1 mmol) in dry *N,N*-dimethylformamide (5 mL) was treated in sequence with a solution of FT49 (957 mg, 3 mmol) in dry *N,N*-dimethylformamide (5 mL), copper iodide (38 mg, 0.2 mmol), triethylamine (0.7 mL, 5 mmol), and palladium tetrakistriphenylphosphine (115 mg, 0.1 mmol). The reaction mixture was stirred for 6 hours at room temperature. The solvents were removed by co-evaporation with toluene under reduced pressure. The residue was dissolved in dichloromethane and washed with aqueous solutions of 5 % Na_2-EDTA (2), 10 % sodium thiosulphate, and water. The organic phase was dried with sodium sulphate and evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel with dichloromethane/methanol (95:5) and subsequently with ethyl acetate/*n*-hexane/ethanol/triethylamine (65:30:5:1) to yield 650 mg (77 %) FT59 as a pale yellow foam.

Confirmation of Identity of FT59

Calculated Atomic Composition: C 60.91, H 4.75, N 4.96; Measured Composition: C 60.73, H 4.74, N 4.93.

^{1}H NMR ($CDCl_3$) 2.31 (m, 1 H), 2.51 (m, 1 H), 3.31–3.66 (m, 6 H), 3.79 (s, 6 H), 4.06–4.15 (m, 3 H), 4.52 (m, 1 H), 6.31 (m, 1 H), 6.84 (d, J = 10 Hz, 4 H), 7.20–7.67 (m, 14 H), 7.87 (m, 2 H), 8.10+8.18 (2 s, 1 H); MS (FAB) *m/z* 848 $[M+H]^+$.

Mass Spectrometry of model linkers FT27 and FT28

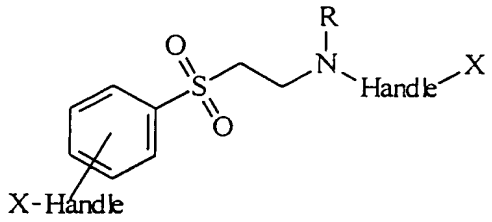
All data was acquired on a Platform-LC quadrupole instrument (Micromass Ltd, UK) with an electrospray ionisation source.

A solution of 5ng/µl of FT28 in a 50:50 mixture of water and ??? was prepared. Ammonia was also present at 0.2%. Fig 8 shows the negative ion mass spectrum of FT28 at a cone voltage of ???. There is a relatively small peak at 380.1 corresponding to the molecular minus a single proton. There is a second much larger peak at 212.1 corresponding to the main cleavage product of FT28 that results from the ionisation of FT28. Figure 9 shows the probable mechanism by which the FT28 linker cleaves.

It should be noted that the trifluoracetate group in FT28 protecting the amine linkage is unstable to strong base which is used in the final deprotection steps in an oligonucleotide synthesiser. This means that a different group must be found to replace this group which is stable to oligonucleotide synthesis to generate a linker that cleaves with high efficiency. Furthermore a reactive functionality must be substituted into the phenyl ring to provide the second handle required for this entity to be useful as a linker group.

Claims:

1. A compound having the following formula:



wherein R is an electron withdrawing substituent, one X comprises a mass marker and the other X comprises one or more nucleic acid bases, and each Handle is the same or different, being either a single bond directly attaching the X groups to the phenyl ring and the amine functionality respectively, or a divalent group capable of attaching the X groups to the phenyl ring and the amine functionality respectively.

2. A compound according to claim 1, wherein R is a hydrogen atom, a halogen atom, or a substituent comprising a carbonyl group and/or a halogen atom.

3. A compound according to claim 2, wherein R is a fluorine atom, a chlorine atom, a bromine atom, an iodine atom, a trfifluoroacetyl group, or a trifluoromethyl acetate group.

4. A compound according to any preceding claim, wherein each Handle is independently a -CO-NH- group, an -NH-CO-NH- group, an -NH-CS-NH- group, a $-CH_2-NH-$ group, an $-SO_2-NH-$ group, an $-NH-CH_2-CH_2-$ group, or an -OP(=O)(O)O- group.

5. A compound according to any preceding claim, wherein the X group comprising one or more nucleic acid bases, comprises a nucleotide, an oligonucleotide or a nucleic acid.

6. A compound according to claim 5, wherein the nucleotide, oligonucleotide or nucleic acid is natural, or is modified by modifying a base, sugar and/or backbone of the nucleotide, oligonucleotide or nucleic acid.

7. A compound according to any preceding claim, wherein the mass marker comprises a polyether.

8. A compound according to claim 7, wherein the polyether is a substituted or unsubstituted poly(arylether).

9. A compound according to claim 7 or claim 8, wherein the polyether comprises one or more fluorine atom substituents.

10. A compound according to any preceding claim, wherein the mass marker comprises a metal ion-binding moiety.

11. A compound according to claim 10, wherein the metal ion-binding moiety is a porphyrin, a crown ether, hexahistidine, or a multidentate ligand.

12. A compound according to claim 11, wherein the metal ion-binding moiety is a bidentate ligand or is EDTA.

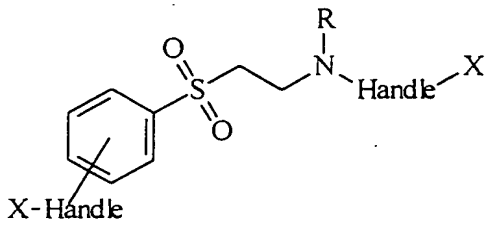
13. A compound according to any of claims 10-12, wherein the metal ion-binding moiety is bound to a monovalent, divalent or trivalent metal ion.

14. A compound according to claim 13, wherein the metal ion is a transition metal ion, or a metal ion of group IA, IIA or IIIA of the periodic table.

15. A compound according to claim 14, wherein the metal ion is Ni^{2+}, Li^{+}, Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, or Al^{3+}.

16. A method for characterising a nucleic acid or other molecule, which method comprises identifying a mass marker by mass spectrometry, which mass marker is relatable to a specific nucleic acid base or base sequence, or a specific atom or group in a molecule, to identify the mass marker and thereby identify the base or base sequence, or the specific atom or group,

wherein the mass marker is attached to the nucleic acid or other molecule by a linker group having the following formula:



wherein R, each X and each Handle are as defined in claim 1.

17. A method according to claim 16, wherein R is as defined in claim 2 or claim 3.

18. A method according to claim 16 or claim 17, wherein Handle is as defined in claim 4.

19. A method according to any of claims 16-18, wherein one X is independently as defined in claim 5 or claim 6.

20. A method according to any of claims 16-19, wherein the mass marker is as defined in any of claims 7-9.

21. A method according to any of claims 16-20, wherein the mass marker comprises a metal ion-binding moiety.

22. A method according to claim 21, wherein the metal ion-binding moiety is as defined in claim 11 or claim 12.

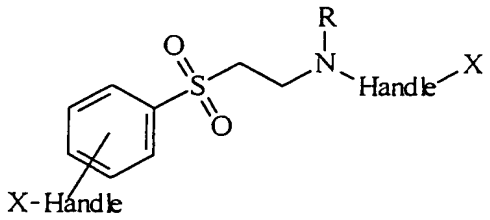
23. A method according to claim 21 or claim 22, which method further comprises binding a metal ion as defined in any of claims 13-15 to the metal ion-binding moiety, prior to identifying the mass marker by mass spectrometry.

24. A method according to any of claims 16-23, which method further comprises forming a compound as defined in any of claims 1-15, prior to identifying the mass marker.

25. A method according to any of claims 16-24, which method further comprises heating the linker group to cleave off the mass marker.

26. A method according to any of claims 16-25, which method further comprises cleaving off the mass marker in the mass spectrometer.

27. Use of a linker group having the following formula:



wherein R, each X and each Handle are as defined in any of claims 1-6, in the characterisation of a nucleic acid or other molecule, by identifying a mass marker by mass spectrometry.

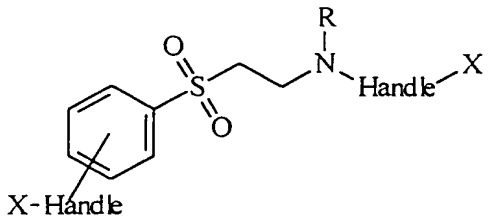
28. Use according to claim 27, wherein the mass marker is as defined in any of claims 7-15.

29. Use according to any of claims 28-30, wherein the mass marker forms part of a compound as defined in any of claims 1-15.

ABSTRACT

COMPOUNDS FOR MASS SPECTROMETRY

Provided is A compound having the following formula:



wherein R is an electron withdrawing substituent, one X comprises a mass marker and the other X comprises one or more nucleic acid bases, and each Handle is the same or different, being either a single bond directly attaching the X groups to the phenyl ring and the amine functionality respectively, or a divalent group capable of attaching the X groups to the phenyl ring and the amine functionality respectively.

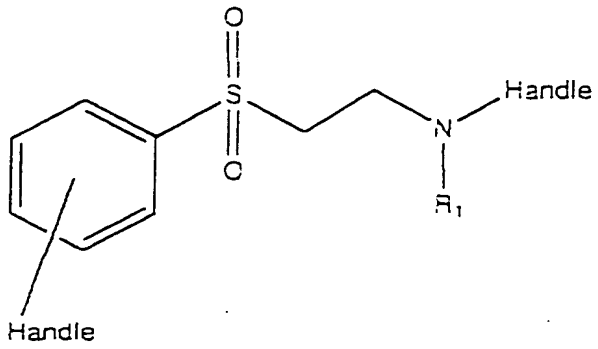
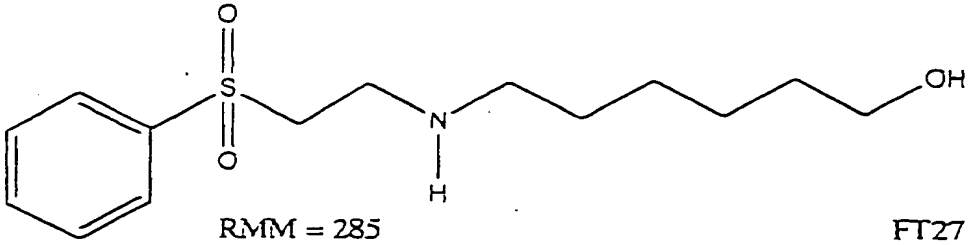


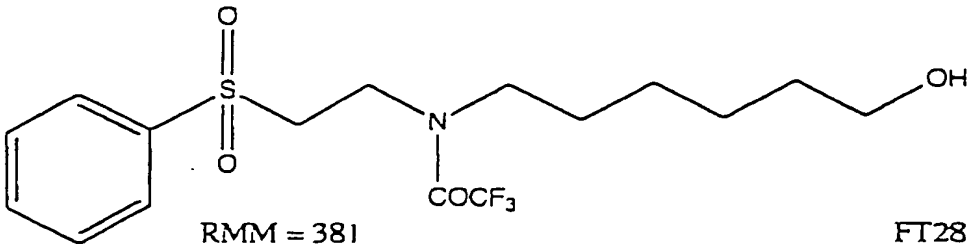
Figure 1



RMM = 285

FT27

Figure 2



RMM = 381

FT28

Figure 3

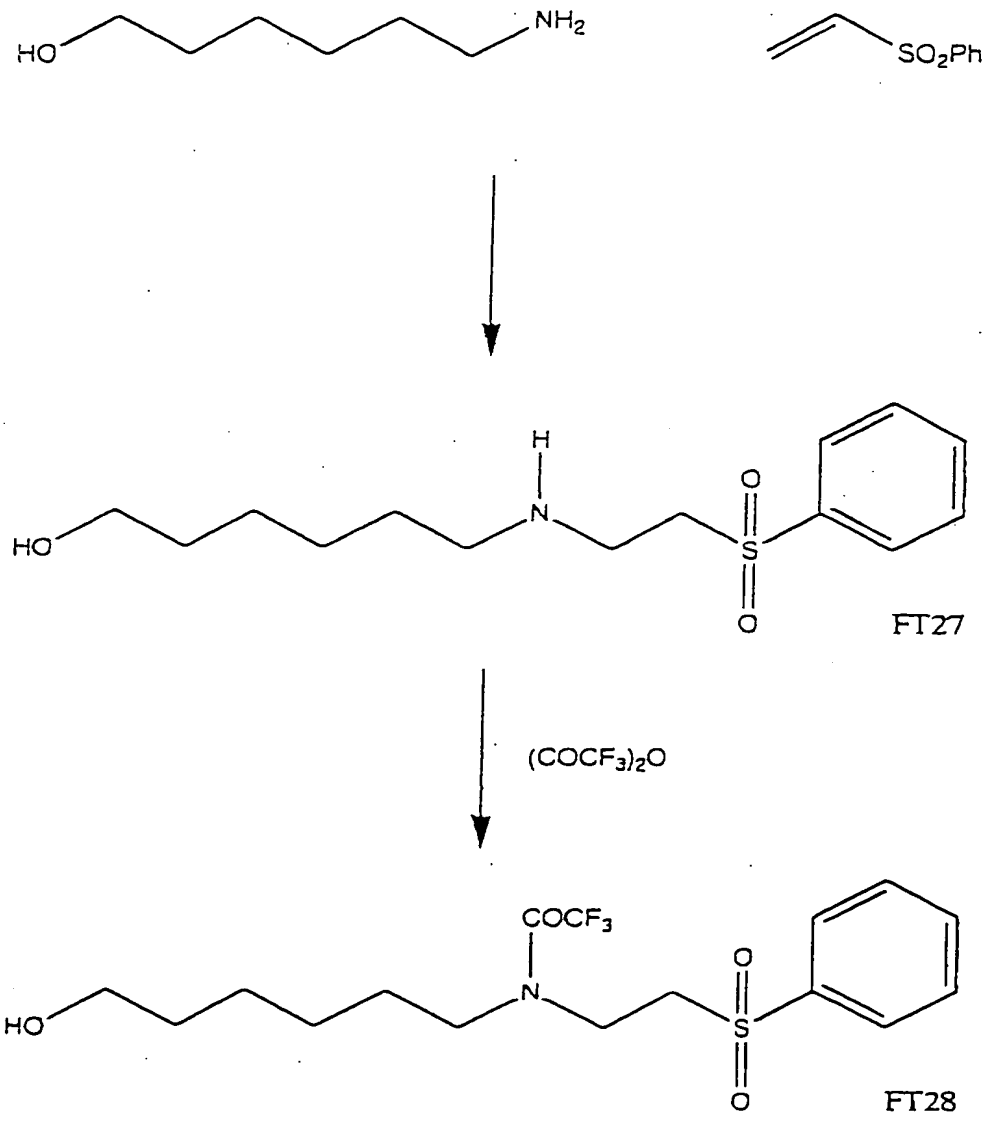


FIGURE 4

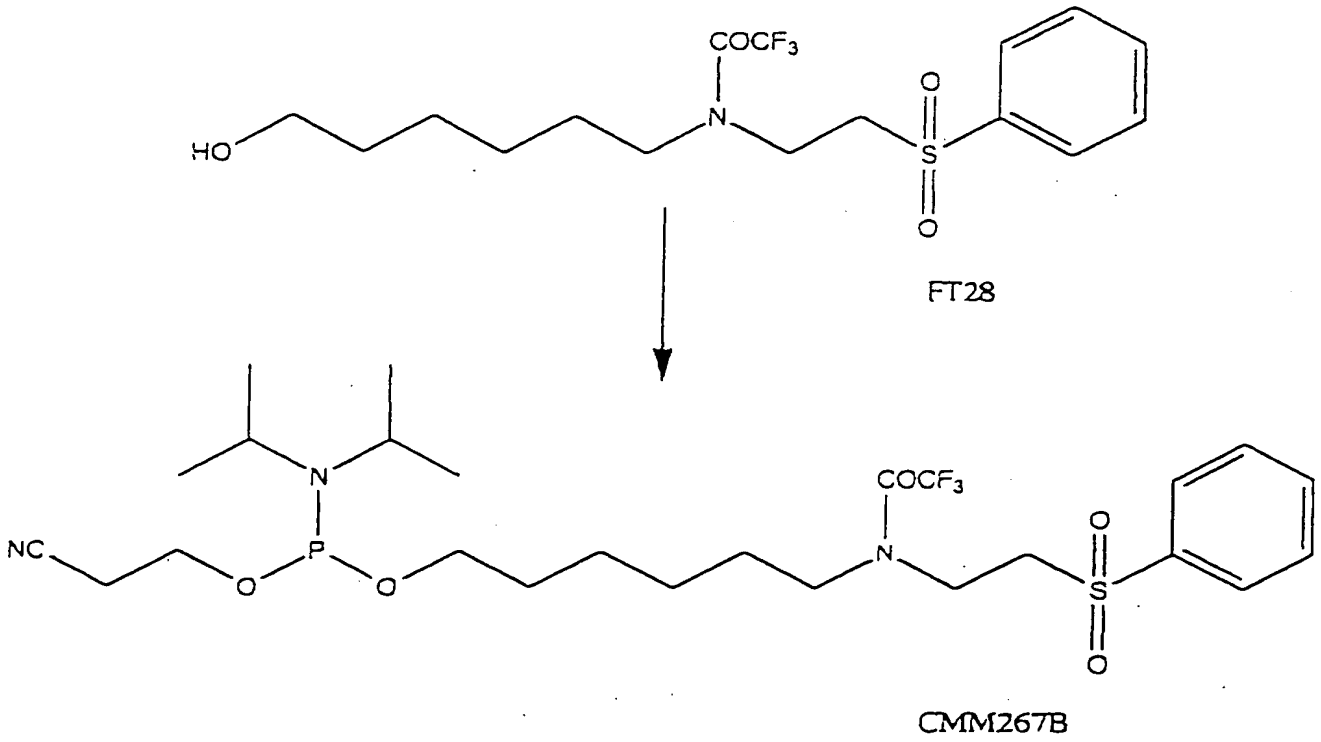


FIGURE 5

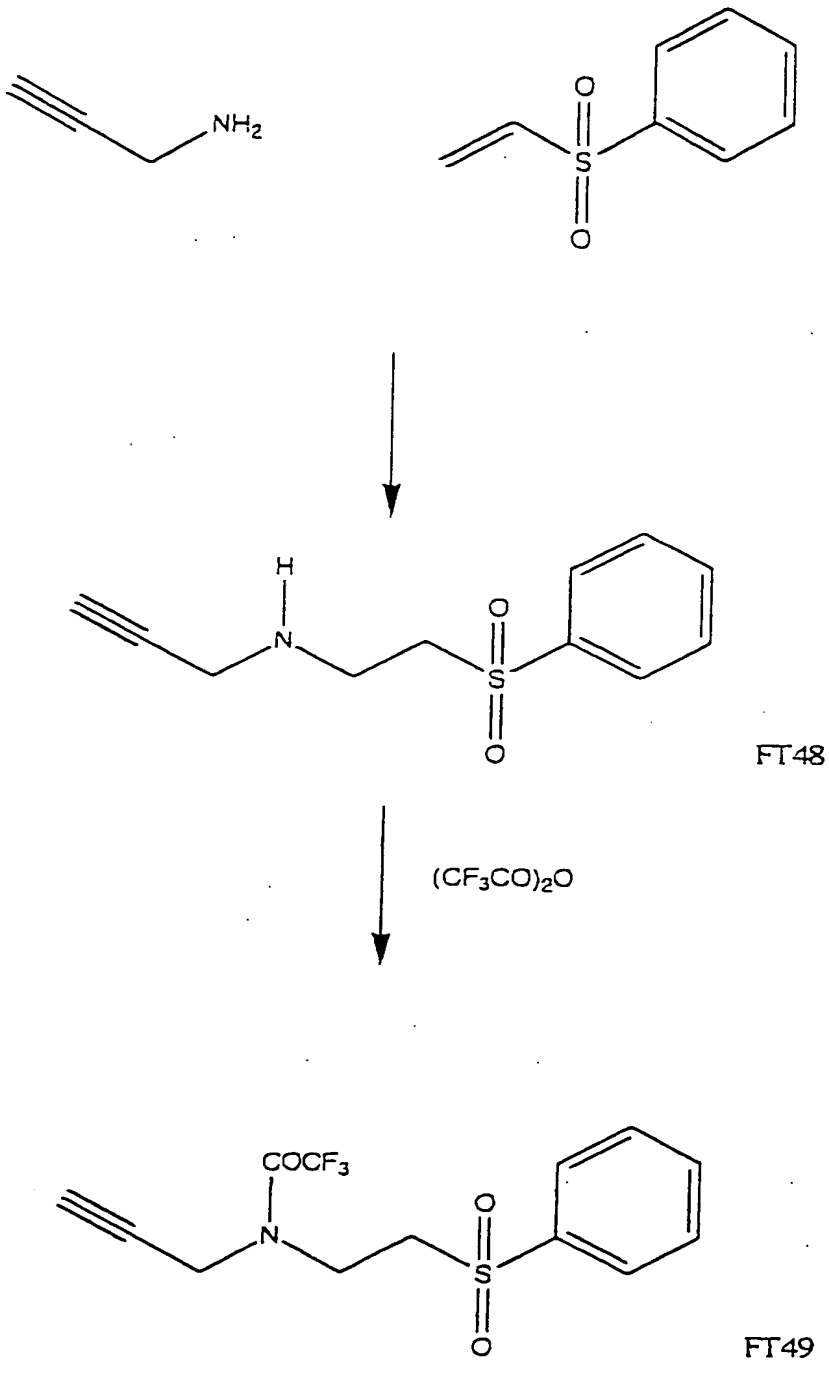
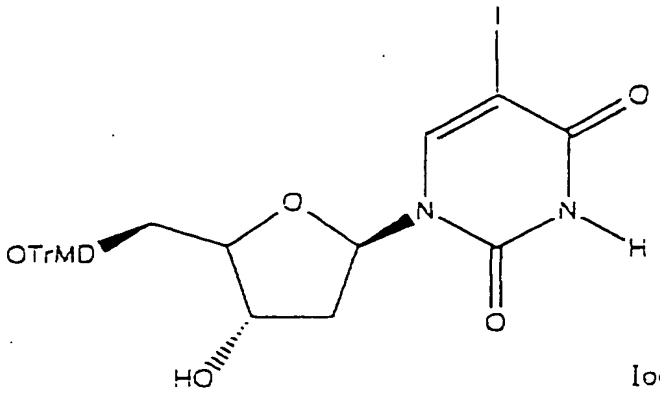
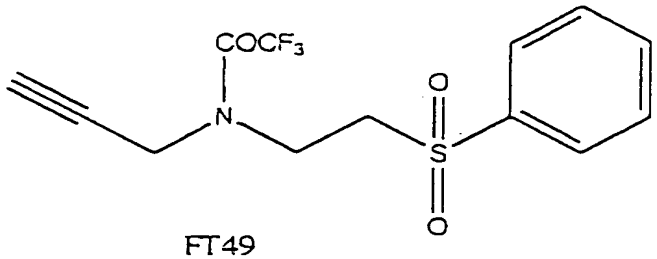


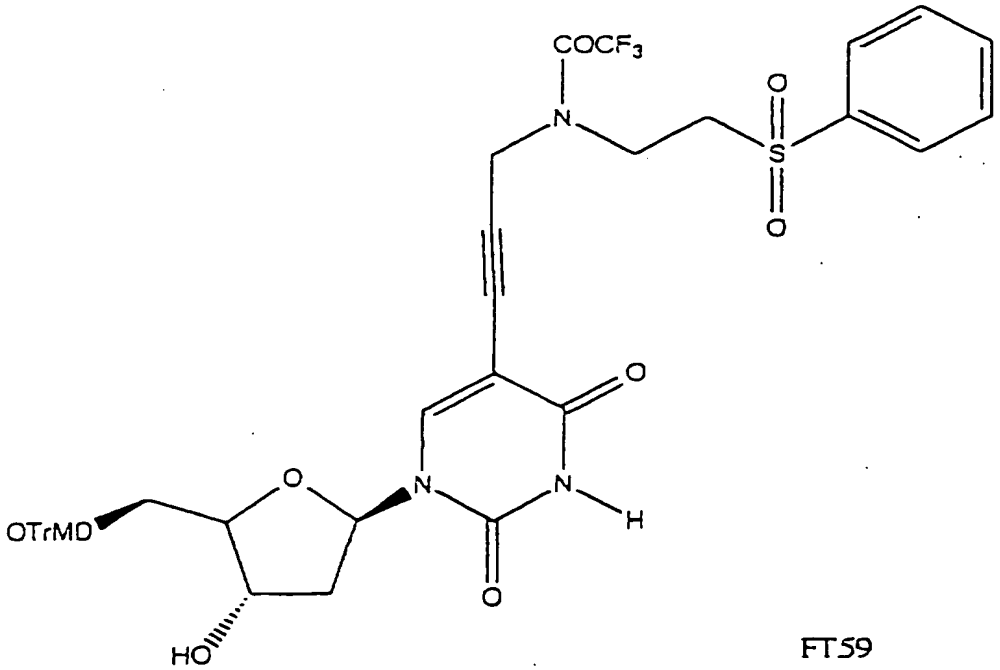
FIGURE 6



Iodouridine



FT49



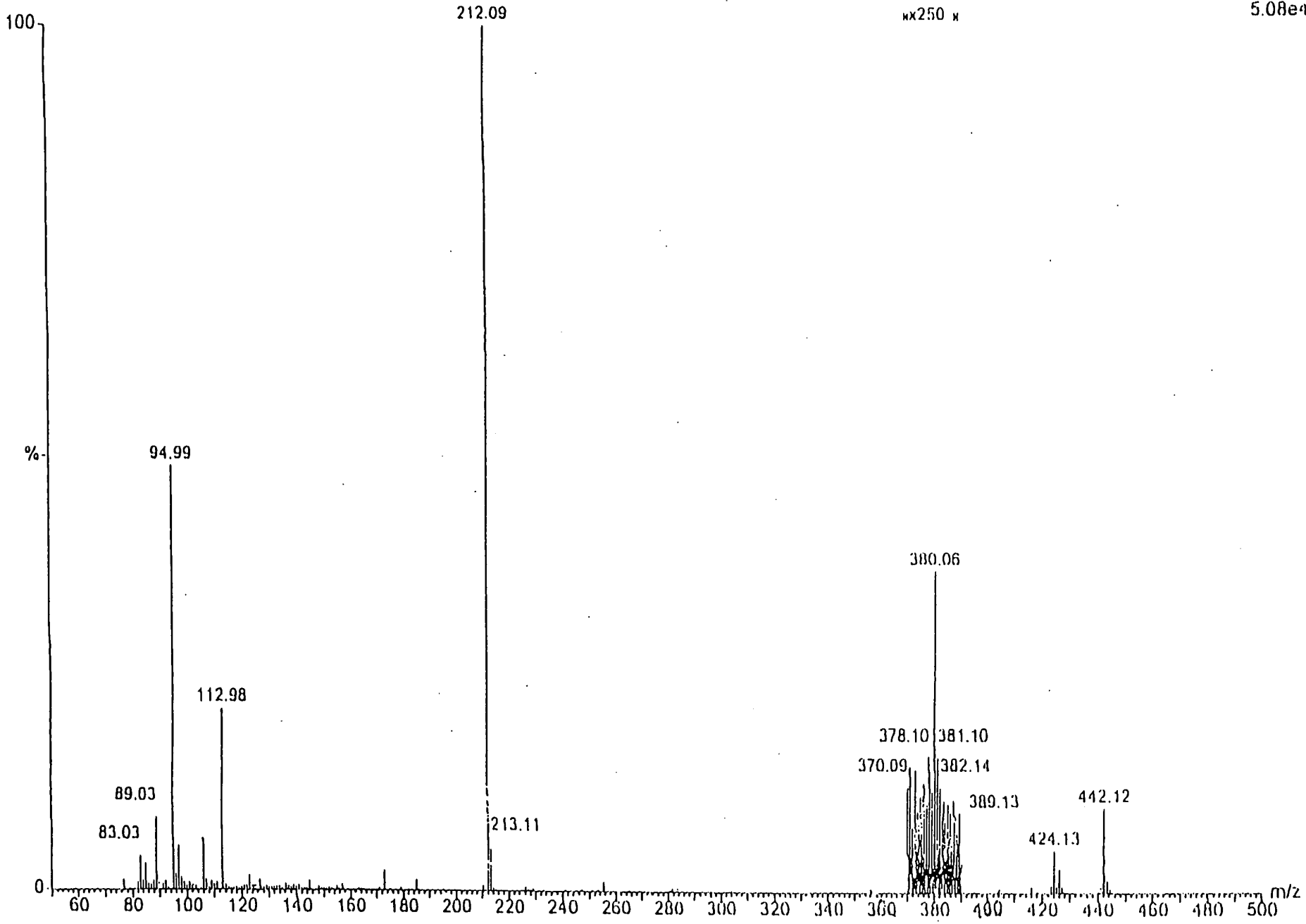
FT59

FIGURE 7

Marker FT28 @ 5ng/uL in ACN:H20 (50:50 v/v) + 0.2% ammonia.
I12802 32 (0.588) Cm (12:104)

FIGURE 8

TOF MS ES
5.08e4



6/7

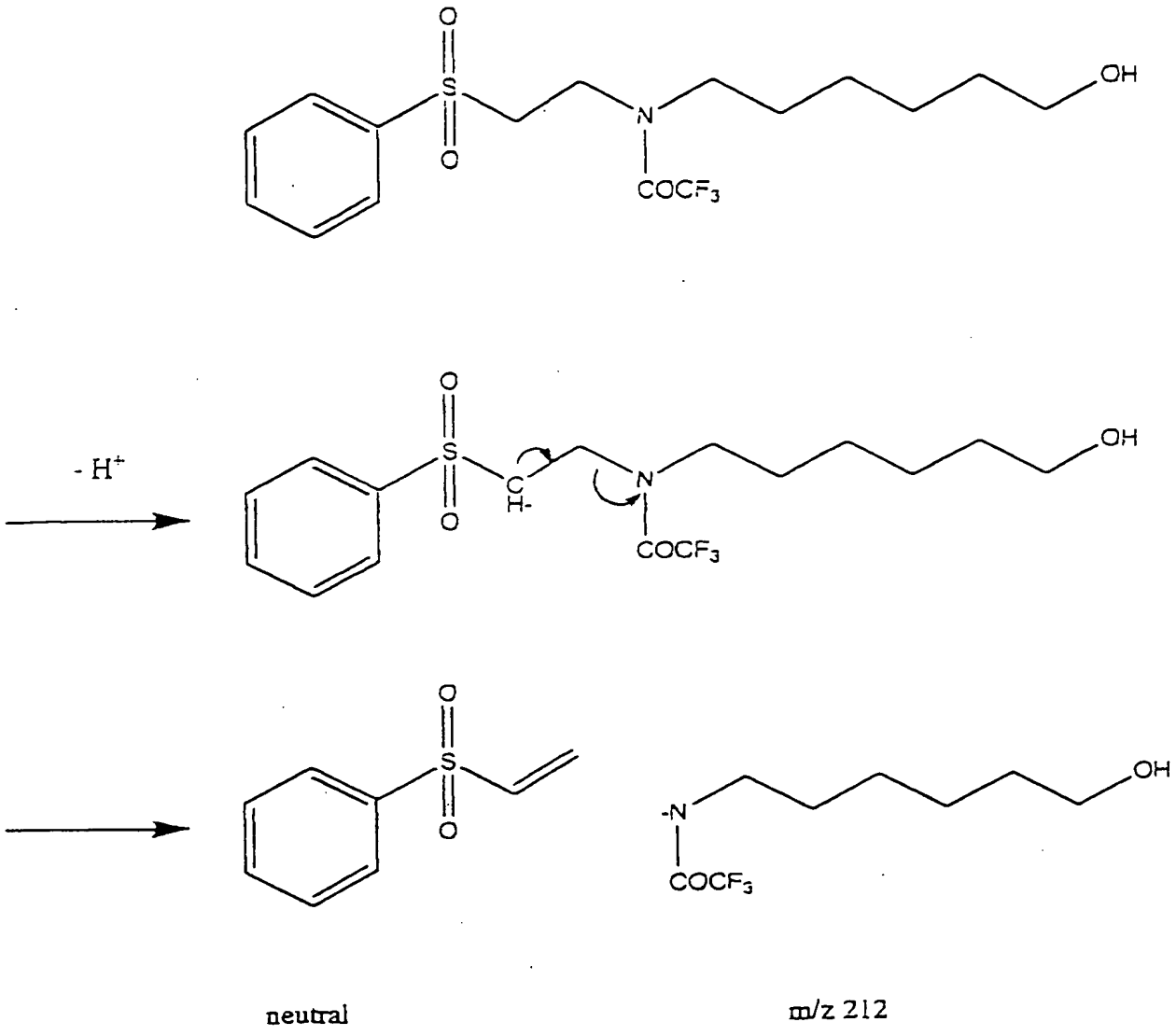


figure 9

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